Research Article

Cancer Prevention Research

Combined Genetic Biomarkers and Betel Quid Chewing for Identifying High-Risk Group for Oral Cancer Occurrence

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Abstract

We integrated genetic risk scores (GRS) and environmental factors for identifying high-risk subjects for oral squamous cell carcinoma (OSCC) occurrence by using case–control study. A total of 447 patients diagnosed with OSCC and 580 unrelated subjects were recruited from two medical centers in Taiwan. A multinomial logistic regression model was conducted to access interaction between GRS and betel quid (BQ) chewing. We employed ROC curve to compare the accuracy of OSCC occurrence. Four tag SNPs were found in *NOTCH1*, *BRCA1*, *COL9A1*, and *HSPA13* genes that were significantly associated with OSCC occurrence. GRS was calculated by the four tag SNP risk alleles. The higher GRS (scores = 4) remained independently associated with risk of OSCC after adjustment for age, the use of alcohol, BQ, and cigarette: adjusted OR = 4.42 [95% confidence interval (95% CI), 1.34–14.55]. The GRS and BQ chewing interaction showed an increased risk for OSCC occurrence with adjusting for other substance use and age (OR = 70.77; 95% CI, 8.70–575.73). The synergy index was 16.58 (95% CI, 2.27–70.56), suggesting a positive additive interaction between GRS and BQ chewing. The areas under the ROC curves (AUROC) were 0.91 for combined GRS and BQ chewing with sensitivity of 88.6% and specificity of 86.7%. The AUROC of GRS and BQ chewing is above 90%, which may be valuable in identifying high-risk subjects. Early screening can allow the clinician to provide the appropriate intervention and to reduce the OSCC occurrence. *Cancer Prev Res; 10(6); 355–62.* *©2017 AACR.*

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Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in the world. The most common type of oral cancer is OSCC, which accounts for approximately 90% of all malignant oral neoplasms (1). The previous studies conducted a survey (from 1975 to 1996) of 378 cancer patients in Taiwan, indicating that the 5-year survival rate for stage III oral cancer was 49%, declining to 30% for stage IV (2). The survival rate for the earliest stage is >75%, which suggests the importance of the early detection for OSCC in reducing morbidity and mortality (3).

This low survival rate has been attributed to the lack of understanding of the etiopathogenesis of OSCC (4, 5). Major risk factors associated with the development of OSCC are genetic risk factors and environmental risk factors, including betel quid (BQ) chewing and cigarette and alcohol consumption (6). Genetic risk factors and environmental risk factors have a synergistic effect on the occurrence of OSCC.

BQ chewing is common in Central Asia. It is estimated that currently 10% of the world population or nearly 700 million individuals chew BQ regularly, and it is thought to be the fourth most commonly used psychoactive substance in the world (7). Research conducted in Taiwan, specifically those employing environmental epidemiology and case–control approaches, had initially confirmed a correlation between the effect of BQ use and oral, pharyngeal, and laryngeal cancer, in addition to precancerous lesions. An additive effect is observed in cases of simultaneous tobacco, alcohol, and betel use, generating a 123 times higher risk of oral cancer (8). The overall attributable fraction for tobacco, alcohol, and BQ chewing suggests a 93% attribution for oral cancer (9).

Genetic changes, such as mutations or overexpression, initiate cell proliferation and migration, which commonly results in the occurrence of cancer. Many studies have investigated the correlation between genetic factors and oral cancer, with numerous genes exhibiting an association with oral cancer. These studies primarily employed cancer cells or cell lines from oral cancer patients to examine the molecular mechanisms of malignant changes or signal transduction during carcinogenesis. Most studies have focused on the p53 tumor suppressor gene; previous studies showed that 38% to 81% of oral cancer in the United States was attributed to p53 alterations (10, 11),

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and that in Taiwan was 5% (12). The Ras oncogene mutation approximated 0% among Caucasians (13, 14), whereas that in Taiwanese was higher at 18%. This discrepancy may be due to exposure to different environmental factors.

The causes of oral cancer have clearly been confirmed. Early detection of primary tumor and relapse is a key factor for improving the survival of patients with OSCC, because high rates of cases are recognized at advanced stages. Consequently, preventative models should be established to effectively eliminate the interaction between exposure to tobacco, alcohol, and betel use and individual susceptibility genes. Genetics is an important risk factor for OSCC occurrence, but it remains unclear whether genetic factors could improve the predictability of OSCC occurrence. Therefore, we integrated the model of genetic profiling with environmental factors to identify high-risk group for oral cancer occurrence.

Materials and Methods

Subjects

A total of 447 patients diagnosed with OSCC were recruited from the Department of Dentistry and the Department of Otorhinolaryngology, Kaohsiung Medical University Hospital (KMUH; Kaohsiung, Taiwan) in the southern Taiwan ($n = 285$) and Changhua Christian Hospital (CCH; Changhua, Taiwan) in mid-Taiwan ($n = 162$). The control groups, composed of 580 unrelated subjects, were frequency matched with OSCCs by gender and were from the same geographic areas as cases. The controls included patients with eye problems (cataract and glaucoma), bone fractures, and subjects undergoing physical checkups from KMUH ($n = 331$) and CCH ($n = 249$). Data on social–demographic factors, anthropometric parameters, smoking, drinking and betel chewing habits, medical history, and current medications were obtained by interviewing the subjects. An individual without substance use of alcohol, betel quid, and cigarettes was defined as nondrinker, nonchewer, and nonsmoker, respectively. Details of patterns of betel quid, alcohol, and tobacco use comprised types consumed, age at initial use, daily consumption, use frequency, years of substance use, and achievement of abstinence (15). This study was approved by the Institutional Review Boards of KMUH and CCH, informed consent committee on human subjects, and biospecimen unitization committee.

Selection of tag SNPs of susceptibility candidate genes

We evaluated and chose susceptibility genes, which have been reported in association between BQ use and development of OSCC. These susceptible genes (16) included *CYP26B1* (17), *MAOA* (18), *S100A12*, inflammation response (*COX-2*, *HSPA13*, and *DNAJA1*; ref. 15), tumor suppressor gene (*FANGG*, *HAF1A*, *HF1B*, *BRCA1*, and *NOTCH1*; refs. 19, 20), extracellular matrix structural (*COL9A1*) and growth differentiation (*GDF15*). Some candidate genes related to OSSC were also included in this study (21). On the basis of linkage disequilibrium patterns of Han Chinese, 50 tag SNPs among 17 genes with an MAF greater than 5% were selected from the HapMap database. More detailed information is shown in Supplementary Table S1.

DNA extraction and genotyping

Genomic DNA was extracted from the peripheral blood samples using the Puregene DNA Isolation Kit (Gentra Systems) according to the manufacturer's instructions. Genotyping of the 50 SNPs was performed using the Sequenom MassARRAY System by the Academia Sinica National Genotyping Center (Taipei, Taiwan). In brief, primers for PCR and iPLEX reaction were designed by Genotyping Tools & Mass ARRAY Assay Design software. Then, the Mass ARRAY iPLEX reaction was performed. After the iPLEX reaction, each base of SNP site will create iPLEX products with different molecular weight. Matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) can identify the different iPLEX product, and we finally obtained the information of each SNP site for every subject after analyzing the result of MALDI-TOF with TYPER 4.0 software.

SNP risk score and combined model risk scores

We calculated an SNP risk score based on estimates of the OR per allele and risk allele frequencies (p) assuming independence of additive risks (22). A score of 1 was given to each A allele of BRCA1 - rs2070833 and each T allele of COL9A1- rs550675, A allele of NOTCH1 - rs139994842 and each T allele of HSPA13 - rs2822641. The overall genetic risk score (GRS) was then calculated by the SNP risk alleles.

Statistical analysis

Statistical analysis in this study was performed using SAS 9.2 software. All genotype frequencies of control population were tested for Hardy–Weinberg equilibrium. The difference between the practical and expected number of each genotype was compared by χ^2 test. Hardy–Weinberg equilibrium was assumed for P value more than 0.05. Student t test was applied to compare the age difference, whereas Pearson χ^2 test or Fisher exact test was used to determine the difference of gender distribution and SNP genotype frequencies between case and control subjects. The OR and the corresponding 95% confidence intervals (95% CI) were assessed by logistic regression. We calculated an SNP risk score using estimates of the OR per allele and risk allele frequencies, assuming independence of additive risks. The GRSs were classified into three categories (0, 1–3, and 4) to appropriately reflect the range of the scores. A multinomial logistic regression model including GRS × BQ use interaction term and adjusted for covariates was applied. Additive interactions were evaluated with the use of a synergy index and their 95% CIs, calculated as: (OR for GRS categories and alcohol use−1) ÷ [(OR for GRS categories + OR for alcohol use)−2] after adjusting for covariates. The attributable fraction (percent) among all cases in the population (AF-Pop %) was estimated (exposure frequency in OSCC cases × (OR−1)/OR). We employed ROC curves to compare the accuracy of occurrence of OSCC. We would like to validate prediction model for subjects recruited from two medical centers. Within the training and validation sets, subjects recruited from KMUH were assigned to the training, and subjects recruited from CCH were assigned to validation analysis sets.

Results

The mean age of the cases and control was 56.6 years (SD, 10.5) and 51.4 years (SD, 13.5), respectively ($P = 0.004$). The majority of participants were males. The proportions of males were 96.2% in the case group and 97.4% in the control group ($P = 0.2658$). Among the 447 cases, 72.3% had the habit of drinking alcohol compared with 25.2% among the 580 controls ($P < 0.0001$).

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Table 1. Comparing characteristics of the study subjects

Variable	Cases $n = 447$	Controls $n = 580$	*P*
Male, *n* (%)	430 (96.2)	565 (97.4)	0.2658
Age, year (SD)	56.6 (10.5)	51.4 (13.5)	0.0004
<40	21 (4.7)	113 (19.5)	
40 ≤ age < 50	101 (22.6)	145 (25)	
50 ≤ age < 60	138 (30.9)	169 (29.1)	
≥60	187 (41.8)	153 (26.4)	
Cigarette, *n* (%)			
None	55 (12.3)	298 (51.4)	
Ever	213 (47.7)	108 (18.7)	
Current	179 (40.0)	174 (30.9)	<0.0001
Alcohol, *n* (%)			
None	124 (27.7)	434 (74.8)	
Ever	191 (42.7)	40 (6.9)	
Current	132 (29.6)	106 (18.3)	<0.0001
BQ chewing, *n* (%)			
None	75 (16.8)	506 (87.2)	
Ever	303 (67.8)	57 (9.8)	
Current	69 (15.4)	17 (2.9)	<0.0001

Table 2. Association between selected SNP and the risk of OSCC occurrence

SNP	Gene	Cases $n = 447$	Controls $n = 580$	OR (95% CI)	*P*
rs2070833	*BRCA1*				<0.0001
CC		237	374	1	
CA		157	175	1.42 (1.09–1.86)	
AA		53	31	2.70 (1.68–4.33)	
rs550675	*COL9A1*				0.0005
CC		187	314	1	
CT		178	180	1.66 (1.26–2.19)	
TT		82	86	1.60 (1.13–2.28)	
rs139994842	*NOTCH1*				0.0006
GG		401	560	1	
AG		34	15	3.17 (1.69–5.89)	
AA		12	5	3.35 (1.17–9.59)	
rs2822641	*HSPA13*				0.0006
GG		386	542	1	
GT		56	36	2.18 (1.41–3.39)	
TT		5	2	3.51 (0.67–3.51)	

Among the 447 cases, 83.2% had the habit of consuming betel quid compared with 12.7% among 580 controls ($P < 0.0001$). Of the 447 cases, 87.7% smoked cigarettes compared with 49.6% among the 580 controls who smoked cigarettes ($P < 0.0001$; Table 1). Comparison characteristics of the study subjects from two medical centers were presented in Supplementary Table S1. Supplementary Table S2 listed the genotype and allele frequencies of individual SNP in case and control subjects. A total of 46 SNPs in 13 genes were not associated with the occurrence of OSCC. We noticed that four SNPs, rs2070833 in *BRCA1* gene, rs550675 in *COL9A1* gene, rs139994842 in the *NOTCH1* gene, and rs2822641 in the *HSPA13*, were significantly associated with the risk of OSCC. Compared with those carrying wild-type genotype and allele, patients carrying variant CA/AA genotype of rs2070833 had an increased risk of OSCC (OR = 1.42; 95% CI, 1.09–1.86; OR = 2.70; 95% CI, 1.086–2.656). The CT/TT genotypes of rs550675 had the same effects on the risk of OSCC (OR = 1.66; 95% CI, 1.26–2.19; OR = 1.60; 95% CI. 1.13–2.28). As for rs139994842, minor genotypes (AG/AA) showed a strong association with higher OSCC susceptibility than the wild-type genotype (OR = 3.17; 95% CI, 1.69–5.89; OR = 3.35; 95% CI, 1.17–9.59). The GT genotype of rs2822641 also exhibited association with OSCC risk (OR = 2.18; 95% CI, 1.14–3.39). However, there was no statistical difference in genotype TT between cases and controls (OR = 3.51; 95% CI, 0.67–3.51; Table 2).

We constructed GRS by using four significant SNPs (Table 2). The univariate associations of conventional risk factors, the use of alcohol, BQ, and cigarette, and GRS associated with OSCC risk are shown in Table 3. The GRS was associated with the risk of OSCC (unadjusted OR ranged from 1.64 to 3.24). The higher GRS remained independently associated with the risk of OSCC after adjustment for age, and the use of alcohol, BQ, and cigarette (adjusted OR = 3.11; 95% CI, 1.21–10.67).

To evaluate the interaction between GRS and the use of BQ chewing, we categorized GRS into ether three categories (GRS = 0, 1 ≤ GRS ≤ 3, and GRS > 3). The distribution of case numbers and genetic scores was provided in Supplementary Table S3. We found that the adjusted OR of GRS increased with consuming quantities of betel nut compared with controls without BQ chewing. Specifically, OSCC cases with carriers of GRS = 4 were increased with occurrence of OSCC in a dose response manner (without BQ chewing adjusted OR = 3.83; 95% CI, 1.07–13.69; moderate dose adjusted OR = 47.44; 95% CI, 5.70–394.369; severe dose adjusted OR = 76.67; 95% CI, 9.39–625.55; Supplementary Table S3). GRS ($P = 0.05$) and BQ chewing ($P < 0.01$) were found to be associated with oral cancer stage (Supplementary Table S4).

Table 4 shows the joint effects of multilocus profiles of GRS and BQ use in OSCC patients. We found that there was a significantly higher risk of OSCC without BQ use in GRS = 4 group (OR = 4.42; 95% CI, 1.34–14.55) and alcohol use in GRS = 4 group (OR = 70.77; 95% CI, 8.70–575.73). The synergy index was 16.58 (95% CI, 2.27–70.56), suggesting a positive additive interaction between GRS and BQ chewing. However, the relatively low frequency in the population of the GRS = 4 (2.24% among controls) and of the joint exposure (0.17%) translates into considerable population attributable fractions for OSCC (2.21% and 0.87%, respectively; Table 4).

Table 3. Predicted risk of oral cancer for environmental factors and genetic scores

Parameters	OR (95% CI)[a]	OR (95% CI)
Age	1.02 (1.01–1.03)	1.03 (1.01–1.05)[b]
Alcohol	7.06 (5.29–9.42)	2.0 (1.39–2.81)[b]
Smoking	6.84 (4.90–9.55)	1.61 (1.05–2.56)[b]
Betel	33.39 (23.61–47.25)	26.71 (16.67–42.80)[b]
GRSs[c]		
0	Reference	Reference
1	1.64 (1.20–2.5)	0.96 (0.60–1.54)[d]
2	1.90 (1.36–266)	1.29 (0.79–2.10)[d]
3	1.59 (0.91–2.78)	1.31 (0.60–2.85)[d]
4	3.24 (1.37–7.65)	3.11 (1.21–10.67)[d]

[a]Statistics corresponding to logistic regression testing association between risk of oral cancer and environmental factors and GRSs in a single parameter model.
[b]Statistics corresponding to logistic regression testing association between risk of oral cancer and environmental factors and GRSs with adjustment for age, alcohol smoking, and betel.
[c]A score of 1 was given to each A allele of BRCA1 - rs2070833 and each T allele of COL9A1- rs550675, A allele of NOTCH1 - rs139994842 and each T allele of HSPA13 - rs2822641.
[d]Statistics corresponding to logistic regression testing association among risk of oral cancer, betel nut quantities, and GRSs after adjustment for age and betel.

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Table 4. Attribute risk for GRS and substance use for oral cancer

		Oral cancer *n* (%)	Control *n* (%)	OR (95% CI)	AF-Exp%	AF-Pop%	Synergy index (95% CI)
Score categories	Betel use						
0	No	26 (5.82)	184 (31.72)	1	—	—	
0	Yes	80 (17.9)	22 (3.79)	25.73 (13.77–48.10)	96.11	17.2	
1–3	No	44 (9.84)	313 (23.87)	1.05 (0.59–1.67)	4.95	0.49	
1–3	Yes	282 (63.09)	52 (53.97)	38.38 (23.14–63.66)	97.39	61.45	
4	No	5 (1.12)	8 (1.38)	4.42 (1.34–14.55)	77.38	0.87	
4	Yes	10 (2.24)	1 (0.17)	70.77 (8.70–575.73)	98.59	2.21	16.58 (2.27–70.56)

NOTE: ORs with 95% CIs and their *P* values were estimated after adjusting for covariates using a multinomial logistic regression model. The synergy index is a test of additive interaction that provides evidence that combined exposures are either superadditive (synergy index > 1), compatible with additive (synergy index = 1), or less than additive (synergy index < 1). The synergy index was calculated as: (OR for GRS and betel use−1) ÷ [(OR for GRS + OR for betel use)−2] after adjusting for covariates.

Abbreviations: AF-Exp [(OR−1)/OR], attributable fraction (percent) among exposed cases; AF-Pop (AF-Exp × % among cases), attributable fraction (percent) among all cases in the population.

The prediction accuracy, measured as AUROC for these models, was applied to the GRS and environmental factors. Validation analysis sets were similar to prediction accuracy for the training analysis set (Table 5). We combined two datasets to show AUC; the areas under the ROC curves (AUC) for GRS were 0.64 with sensitivity of 67.55% and specificity of 55.22%. The AUC for BQ chewing was 0.88 with sensitivity of 85.8% and specificity of 84.9% (Table 5). The addition of the GRS showed slightly significant improvement in the sensitivity and specificity (sensitivity, 88.60%; specificity, 86.7%, respectively; Fig. 1). However, the sensitivity and specificity were not significantly different between early stages (stage I and II) and advance stages (stage III and IV; Supplementary Fig. S1).

Discussion

The major findings of our study are: (i) a GRS based on four susceptibility SNPs was associated with occurrence of OSCC independent of environmental risk factors; (ii) for the risk prediction models, combined higher GRS and environmental factors highly predicted OSCC occurrence. Furthermore, we showed risk prediction models to identify individuals with high risk of OSCC occurrence. The combined determination of environmental factors and GRS had the highest sensitivity, specificity, and accuracy compared with only environmental factors.

We found that four tag SNPs, rs139994842 in *NOTCH1* gene, rs2070833 in *BRCA1* gene, rs550675 in *COL9A1* gene, and rs2822641 in the HSPA13, were significantly associated with risk of OSCC. So far, few studies have addressed the association of polymorphisms in NOTCH1 loci with oral cancer susceptibility. Our study demonstrates for the first time the association of the synonymous SNP rs139994842 in exon 14 of NOTCH1 with risk of OSCC occurrence. Although this SNP did not cause amino acid changes, it was located in EGF-like domains of the NOTCH1 extracellular region that may play a dual role in tumorigenesis to regulate various oncogenes and tumor suppressor genes. Both overexpression and downregulation of NOTCH and ligands have been implicated in several human cancers (23–26). Somatic mutations of NOTCH1 have been suggested to play an oncogenic role in breast, colorectal, and pancreatic cancers (27–30). To date, many authors have investigated the role of *NOTCH1* gene

Table 5. Descriptive values of the ROC, sensitivity and specificity for environmental factors and GRSs

Dataset	Parameters	AUC (95% CI)	Sensitivity (%)	Specificity (%)
Training set[a]	GRS	0.61 (0.58–0.66)	83.3	33.1
Training set[a]	Alcohol	0.72 (0.69–0.76)	70	74.5
Training set[a]	Betel	0.86 (0.83–0.89)	85.9	84.9
Training set[a]	Cigarette	0.69 (0.67–0.73)	89.6	50.4
Training set[a]	Age	0.54 (0.51–0.68)	25.6	73.5
Training set[a]	Betel + GRS	0.89 (0.86–0.91)	86.7	86
Validation set[b]	GRS	0.63 (0.57–0.68)	61.6	66.9
Validation set[b]	Alcohol	0.78 (0.73–0.81)	80.8	74.5
Validation set[b]	Betel	0.86 (0.83–0.89)	85.6	86.7
Validation set[b]	Cigarette	0.71 (0.68–0.74)	91.1	50.4
Validation set[b]	Age	0.72 (0.69–0.77)	67.8	73.5
Validation set[b]	Betel + GRS	0.88 (0.84–0.91)	86.3	86.5
Combined[c]	GRS	0.64 (0.57–0.71)	67.55	55.22
Combined[c]	Alcohol	0.73 (0.68–0.79)	74.04	73.63
Combined[c]	Betel	0.88 (0.84–0.92)	85.8	84.9
Combined[c]	Cigarette	0.7 (0.68–0.73)	83.89	58.76
Combined[c]	Age	0.56 (0.48–0.61)	67.1	45.6
Combined[c]	Betel + GRS	0.91 (0.87–0.95)	88.7	86.7

NOTE: The optimal sensitivity and specificity were set with the maximum of AUC.

[a]OSCC and control were recruited from the southern Taiwan ($n = 602$).

[b]OSCC and control were recruited from mid-Taiwan ($n = 425$).

[c]Combined the training set and validation set.

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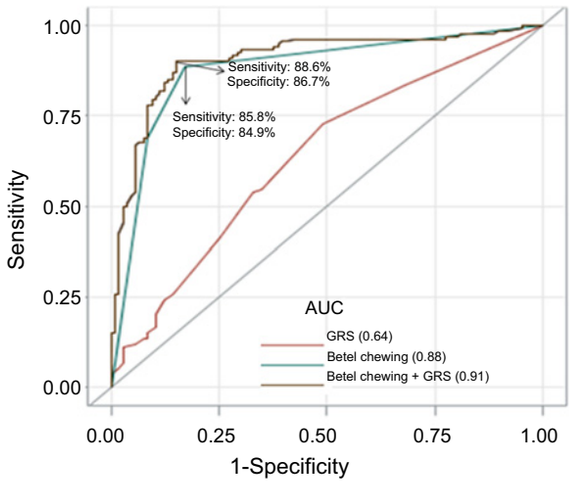

Figure 1.
Receiver operating characteristic (ROC) curves and comparison of areas under the ROC curves for the GRS and BQ chewing were used to predict OSCC occurrence.

polymorphisms in disease pathogenesis. Cao and colleagues found that TC genotype of rs3124591 was positively associated with the risk of development of invasive ductal breast carcinoma in Chinese population (31). Previous reports have demonstrated the association between NOTCH1 rs3124591 and bicuspid aortic valve (32).

BRCA1 gene was shown to be associated with risk of breast, ovarian, and also other cancers (33). BRCA1 seems to have a wide range of cellular processes, such as DNA repair, chromatin remodeling, and transcriptional regulation. Variations in BRCA1 can be classified as rare high-risk mutations, rare moderate-risk mutations, and common low-risk variants; for example, the moderate-risk mutation *BRCA1* p.Arg1699Gln confers a high (40%–80%) lifetime risk of breast cancer occurrence (34). The genetic SNPs were major for examining associations between BRCA1 and breast cancer. Carriage of the risk allele at these loci typically confers between a 1.04- and 1.40-fold increase or a 0.75- and 0.95-fold decrease in breast cancer risk (35).

To the best of our knowledge, this study is the first study to report the genetic association between HSPA13 and COL9A1 and OSCC occurrence. COL9A1 encodes one of the three alpha chains of type IX collagen. Studies have shown that a general drop of methylation in *COL9A1* was apparent in breast tumor tissue (36). HSPA13 is a member of the Hsp70 family of ATPase HSPs. Cancer cells need more HSPs for proper substrate–protein folding on oncogenic pathways (37). Further studies are needed to better understand the role of HSPA13 and COL9A1 in OSCC development. We showed that an interaction between additive GRS and BQ chewing has strong and graded associations with occurrence of OSCC in case and control study.

Although the genetic risk conferred by the individual common genetic variants was modest, their combination resulted in a large effect on the occurrence of OSCC. However, the frequencies of higher GRS were rare, suggesting that variants with large effect sizes will impact a small proportion of the population. Individual risk variants were associated with up to a 77.38% increased risk of developing occurrence of OSCC, with effect sizes similar to that of known environmental risk factors (BQ chewing).

Our GRS was associated with up to a 3.11-fold increased risk of OSCC occurrence, suggesting that combined BQ chewing and GRS may help identify individuals at risk for developing OSCC before the onset of clinical disease.

Most OSCCs are detected in advanced stages, resulting in poor survival rates. Therefore, early OSCC detection is imperative where treatment in the preinvasive stage offers the best prognosis and even the chance of cure. Sensitivity and specificity of different screening tools, such as visual inspection, inspection with methylene blue application, and mouth self-examination, varied and ranged from 18% to 91.4% (38). These screen tools are subjective, relatively unreliable, with inter- and intraobserver variability, hard to validate, require experts, and are expensive to use during screening programs. Consequently, there is an urgent need for precise and convenient diagnostic tools for earlier detection of oral cancer. This is the first study to integrate GRS and environmental factors to detect risk of OSCC occurrence early. When this model is used for risk assessment in asymptomatic individuals, those who test at high risk score may consider several preventive strategies. The most common strategy is increased surveillance, which includes annual visual inspection by clinician and intervention in quitting BQ use. Addiction treatment is complex and extremely difficult; thus, relapse is common. Intervention has adopted a transtheoretical model to combine counseling and medicine treatment for quitting BQ use. The efficacy of prediction model may be useful in identification of different risk groups to suggest intervention and would be increasingly important in the prevention and diagnosis of OSCC.

The strengths of our study include comprehensive environmental exposure information and utilization of multivariate logistic regression analysis to assess the combinatory effect of four genetic polymorphisms for the risk of developing OSCC. However, there are some limitations to our study. The false negative and false positive predictive values were 12.6% and 17.2%, respectively. Among the 73 cancer cases without BQ chewing, 64 may be correctly classified as cancers and nine cancer cases may be misclassified as cancer group by this model. Because the prevalence of BQ chewing in general population is lower compared with this study, application of the risk model to general population in which 90% of them do not use BQ will decrease positive predictive value for detecting OC occurrence. The genes used in this study were predominantly identified by cell model susceptible to alkaloids. The AUC for GRS was 0.64 with sensitivity of 67.55% and specificity of 55.22%. The sensitivity and specificity for GRS were relatively lower compared with the environmental factors. We needed to identify more genes associated with OSCC to increase accuracy in the future study. Because of the low frequency of GRS and high proportion of BQ use in this study, we have suggested that large-scale studies are warranted to further confirm this prediction model used practically in general population without BQ chewing.

Conclusions

In summary, our study provided evidence that GRS based on four susceptibility SNPs was associated with OSCC occurrence.

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For the risk prediction models, combined higher GRS and environmental factors, such as BQ use, may identify high-risk group for oral cancer. Early screening to identify high-risk subjects can allow the clinician to provide appropriate intervention and to reduce OSCC occurrence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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